# SUPPORTING INFORMATION

# Design and characterization of chemically-stabilized Aβ42 oligomers

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Running Title: Scanning PICUP of Aβ

**Table S1.** Occurrence frequencies of WT Aβ42 oligomers of specific order determined using the Cleveland gel method. Values are percent purity ± S.D. Bolded values are those for gel bands that displayed molecular weights matching those expected for oligomers of the nominal oligomer order loaded into the gel.

| | Nominal Oligomer Order | | | | | | |
|---|---|---|---|---|---|---|---|
| **Gel band** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **Monomer** | **79 ± 5** | 19 ± 5 | 13.1 ± 3.9 | 8.3 ± 2.8 | 2.4 ± 1 | 2.3 ± 1.3 | 2.9 ± 0.8 |
| **Dimer** | 2.7 ± 0.3 | **67 ± 3** | 18.5 ± 0.9 | 15.5 ± 0.4 | 10.7 ± 0.3 | 5.1 ± 0.9 | 5.5 ± 0.8 |
| **Trimer** | 10.1 ± 3 | 2.7 ± 0.7 | **43 ± 1** | 18.6 ± 2.6 | 19.5 ± 1.5 | 22.2 ± 2.5 | 12.8 ± 2.7 |
| **Tetramer** | 8.3 ± 2 | 7.2 ± 1.3 | 11.2± 3.5 | **28 ± 2** | 4.6 ± 2 | 4.5 ± 2.1 | 3.8 ± 1.6 |
| **Pentamer** | --- | 3 ± 1.4 | 8 ± 0.8 | 17.5 ± 2.2 | **42 ± 3** | 7.2 ± 0.8 | 10.4 ± 1.7 |
| **Hexamer** | --- | 1.3 ± 0.6 | 5.6 ± 0.9 | 8.9 ± 0.5 | 13.3 ± 2 | **52 ± 4** | 12 ± 1.4 |
| **Heptamer** | --- | 1 ± 0.5 | --- | 3.1 ± 0.6 | 6 ± 0.3 | 4.8 ± 2.3 | **51 ± 4** |
| **Octamer** | --- | --- | --- | 0.4 ± 0.2 | 1.5 ± 0.6 | 2 ± 0.9 | 2.4 ± 2 |
| **≥Nonamer** | --- | --- | --- | --- | 0.3 ± 0.1 | 0.7 ± 0.2 | 0.2 ± 0.2 |

**Table S2.** Occurrence frequencies of [Tyr[1], Phe[10]]Aβ42 oligomers of specific order determined using the Cleveland gel method. Values are percent purity ± S.D. Bolded values are those for gel bands that displayed molecular weights matching those expected for oligomers of the nominal oligomer order loaded into the gel.

| | **Nominal Oligomer Order** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Gel band** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **Monomer** | **93 ± 1** | 16 ± 2.9 | 12.7 ± 3.9 | 5 ± 3 | 2.6 ± 1.3 | 2.8 ± 0.9 | 4.6 ± 1.2 |
| **Dimer** | 3.4 ± 0.4 | **74 ± 3** | 29.7 ± 9 | 20.6 ± 9.1 | 11 ± 3.5 | 7.2 ± 2 | 7 ± 3.1 |
| **Trimer** | 2.6 ± 0.8 | 0.5 ± 0.4 | **36 ± 8** | 19.1 ± 5.8 | 21 ± 1.7 | 18.9 ± 7.1 | 11.5 ± 5 |
| **Tetramer** | 1.5 ± 0.5 | 5.6 ± 2.2 | 10.5 ± 8.2 | **28 ± 5** | 6.1 ± 2.6 | 6.9 ± 3.4 | 8.7 ± 5.9 |
| **Pentamer** | --- | 2.2 ± 1.6 | 6.6 ± 3.8 | 13.7 ± 2.3 | **43 ± 6** | 9.9 ± 3.2 | 5 ± 3 |
| **Hexamer** | --- | 1.9 ± 0.8 | 4 ± 3 | 5.4 ± 1.7 | 12.3 ± 4.2 | **36 ± 1** | 8.2 ± 3 |
| **Heptamer** | --- | --- | 0.2 ± 0.1 | 6.9 ± 5 | 1.8 ± 1.4 | 16.6 ± 9.5 | **52 ± 8** |
| **Octamer** | --- | --- | --- | 1.2 ± 0.9 | 0.7 ± 0.3 | 1.7 ± 0.8 | 3.9 ± 1.7 |
| **≥Nonamer** | --- | --- | --- | --- | 1.2 ± 1 | 0.4 ± 0.3 | --- |

**Table S3.** Occurrence frequencies of [Phe$^{10}$, Tyr$^{20}$]Aβ42 oligomers of specific order determined using the Cleveland gel method. Values are percent purity ± S.D. Bolded values are those for gel bands that displayed molecular weights matching those expected for oligomers of the nominal oligomer order loaded into the gel.

| | **Nominal Oligomer Order** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Gel band** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **Monomer** | **74 ± 3** | 17.7 ± 4.3 | 20.3 ± 6.7 | 6.1 ± 0.5 | 1.9 ± 0.1 | 1.1 ± 0.2 | 1.1 ± 0.2 |
| **Dimer** | 4.3 ± 2.9 | **64 ± 5** | 27.4 ± 1.2 | 20.1 ± 1.4 | 9.4 ± 0.3 | 4 ± 0.8 | 3.1 ± 2 |
| **Trimer** | 11.4 ± 3.3 | 5.5 ± 1.6 | **34 ± 6** | 11.4 ± 1.2 | 9.8 ± 1 | 9.7 ± 3 | 3.8 ± 0.6 |
| **Tetramer** | 9.7 ± 2.2 | 8.2 ± 1.7 | 13.6 ± 3.2 | **41 ± 2** | 5.3 ± 1.9 | 0.2 ± 0.2 | 0.7 ± 0.6 |
| **Pentamer** | --- | 2.5 ± 1.6 | 3.7 ± 1.8 | 13.7 ± 2.4 | **50 ± 2** | 10.5 ± 1.7 | 9.4 ± 2.6 |
| **Hexamer** | --- | 1.9 ± 1 | 1.3 ± 0.6 | 7 ± 0.9 | 14.2 ± 1.3 | **63 ± 2** | 10.9 ± 3 |
| **Heptamer** | --- | --- | --- | 1 ± 0.6 | 5.5 ± 0.9 | 7.3 ± 3.1 | **60 ± 2** |
| **Octamer** | --- | --- | --- | --- | 2.5 ± 0.6 | 2.6 ± 1.7 | 11.6 ± 1.3 |
| **≥Nonamer** | --- | --- | --- | --- | 1.5 ± 0.3 | 1.9 ± 0.8 | --- |

**Table S4.** Occurrence frequencies of [Phe$^{10}$, Tyr$^{30}$]Aβ42 oligomers of specific order determined using the Cleveland gel method. Values are percent purity ± S.D. Bolded values are those for gel bands that displayed molecular weights matching those expected for oligomers of the nominal oligomer order loaded into the gel.

| | **Nominal Oligomer Order** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Gel band** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **Monomer** | **79 ± 2** | 14 ± 7.4 | 8.9 ± 4.2 | 2.7 ± 0.9 | 1.5 ± 0.5 | 1.4 ± 0.4 | 2.4 ± 1.2 |
| **Dimer** | 6.5 ± 1 | **75 ± 7** | 24 ± 3.5 | 17.4 ± 2.3 | 6.1 ± 1.8 | 6 ± 2.9 | 4.6 ± 1.8 |
| **Trimer** | 7.4 ± 1.6 | 5.1 ± 0.6 | **46 ± 5** | 14.6 ± 0.5 | 9.5 ± 2.1 | 3.2 ± 0.8 | 3.5 ± 0.6 |
| **Tetramer** | 6.7 ± 1.6 | 6.5 ± 2 | 16.4 ± 1.5 | **51 ± 4** | 2.6 ± 1.2 | 2.8 ± 1.2 | 2.7 ± 0.7 |
| **Pentamer** | --- | --- | 4.3 ± 1.6 | 10 ± 1.4 | **71 ± 3** | 5.9 ± 0.5 | 3.5 ± 1.5 |
| **Hexamer** | --- | --- | 0.5 ± 0.2 | 2.4 ± 1.1 | 5.8 ± 3.4 | **77 ± 3** | 7.2 ± 1.3 |
| **Heptamer** | --- | --- | --- | --- | --- | 3.7 ± 1.7 | **76 ± 2** |
| **Octamer** | --- | --- | --- | 1.5 ± 0.7 | --- | --- | --- |
| **≥Nonamer** | --- | --- | --- | --- | 3.4 ± 0.9 | 0.7 ± 0.6 | 0.5 ± 0.4 |

**Table S5.** Occurrence frequencies of [Phe$^{10}$, Tyr$^{42}$]Aβ42 oligomers of specific order determined using the Cleveland gel method. Values are percent purity ± S.D. Bolded values are those for gel bands that displayed molecular weights matching those expected for oligomers of the nominal oligomer order loaded into the gel.

| | Nominal Oligomer Order | | | | | | |
|---|---|---|---|---|---|---|---|
| **Gel band** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **Monomer** | **72 ± 1.5** | 7.3 ± 2.3 | 2.1 ± 0 | 1 ± 0.2 | 0.7 ± 0.3 | 0.8 ± 0.4 | 0.2 ± 0.1 |
| **Dimer** | 9 ± 1.2 | **82 ± 0.9** | 7.9 ± 1.4 | 5.1 ± 1.2 | 1.7 ± 0.5 | 0.9 ± 0.2 | 0.8 ± 0.4 |
| **Trimer** | 14 ± 3.6 | 2.3 ± 1 | **81 ± 2.3** | 10.9 ± 0.4 | 2.7 ± 0.6 | 2.6 ± 1.1 | 2.5 ± 0.7 |
| **Tetramer** | 5 ± 2.2 | 8.5 ± 2.5 | --- | **75 ± 2.6** | 1.8 ± 1.4 | 2.4 ± 1 | 2.4 ± 1.3 |
| **Pentamer** | --- | --- | 0.7 ± 0.6 | --- | **90 ± 0.6** | --- | 0.9 ± 0.7 |
| **Hexamer** | --- | --- | 7.6 ± 1.7 | 1 ± 0.8 | --- | **92 ± 1.7** | ---- |
| **Heptamer** | --- | --- | --- | --- | --- | --- | **92 ± 1.2** |
| **Octamer** | --- | --- | --- | 7.1 ± 1.4 | --- | --- | --- |
| **≥Nonamer** | --- | --- | 0.6 ± 0.5 | --- | 3.2 ± 0.2 | 1.8 ± 1.4 | 1.5 ± 1.2 |

**Table S6.** Occurrence frequencies of WT Aβ42 oligomers of specific order determined using two consecutive Cleveland gels. Values are percent purity ± S.D. Bolded values are those for gel bands that displayed molecular weights matching those expected for oligomers of the nominal oligomer order loaded into the gel.

| | **Nominal Oligomer Order** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Gel band** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **Monomer** | **96 ± 2** | 1.1 ± 0.6 | 0.4 ± 0.3 | 0.7 ± 0.5 | 0.5 ± 0.4 | --- | --- | --- |
| **Dimer** | 0.2 ± 0.2 | **90 ± 2** | 2.9 ± 0.7 | 11.7 ± 1.4 | 5.2 ± 0.4 | 0.6 ± 0.3 | 0.4 ± 0.3 | --- |
| **Trimer** | 2.6 ± 1.4 | 0.4 ± 0.3 | **90 ± 2** | 6.1 ± 1.3 | 8.3 ± 0.6 | 10.2 ± 1.2 | 5.1 ± 0.6 | 2.9 ± 1.8 |
| **Tetramer** | 1.2 ± 0.7 | 4.0 ± 1.4 | 2.2 ± 0.3 | **61 ± 5** | 3.3 ± 1.5 | 2.7 ± 0.5 | 5.2 ± 2.6 | 0.8 ± 0.5 |
| **Pentamer** | --- | --- | 0.9± 0.7 | 8.6 ± 3.7 | **59 ± 5** | 2.6 ± 0.4 | 1.3 ± 0.6 | 1.0 ± 0.9 |
| **Hexamer** | --- | 5.0 ± 2.6 | 2.9 ± 0.6 | 8.8 ± 2.8 | 10.7 ± 1.6 | **57 ± 4** | 5.1 ± 1.5 | 5.7 ± 3.9 |
| **Heptamer** | --- | --- | 0.4 ± 0.3 | 0.6 ± 0.5 | 2.4 ± 1.3 | 15.7 ± 3.0 | **64 ± 5** | 2.0 ± 0.9 |
| **Octamer** | --- | --- | --- | 1.9 ± 1.3 | 2.4 ± 0.1 | 5.1 ± 0.8 | 9.1 ± 1.7 | **83 ± 2** |
| **≥Nonamer** | --- | --- | --- | 0.6 ± 0.4 | 3.2 ± 0.4 | 5.9 ± 0.6 | 9.8 ± 6.8 | --- |

**Table S7.** Occurrence frequencies of [Phe$^{10}$, Tyr$^{42}$]Aβ42 oligomers of specific order determined using two consecutive Cleveland gels. Values are percent purity ± S.D. Bolded values are those for gel bands that displayed molecular weights matching those expected for oligomers of the nominal oligomer order loaded into the gel.

| | **Nominal Oligomer Order** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Gel band** | **1** | **2** | **3** | **4** | **5** | **6** | **7** |
| **Monomer** | **96 ± 0.7** | 2.0 ± 1.1 | 1.7 ± 0.9 | 0.6 ± 0.3 | --- | --- | --- |
| **Dimer** | 2.3 ± 1.2 | **96 ± 1.4** | 8.5 ± 2.0 | 4.0 ± 0.7 | 0.7 ± 0.3 | 0.2 ± 0.2 | 0.4 ± 0.3 |
| **Trimer** | 1.0 ± 0.4 | 0.5 ± 0.4 | **87 ± 0.4** | 6.7 ± 0.5 | 3.0 ± 0.6 | 0.9 ± 0.4 | 0.8 ± 0.6 |
| **Tetramer** | 0.5 ± 0.2 | 1.8 ± 0.4 | --- | **83 ± 3** | 3.9 ± 2.1 | 2.4 ± 0.2 | 1.1 ± 0.9 |
| **Pentamer** | --- | --- | 1.3 ± 0.5 | 2.0 ± 1.6 | **91 ± 2.3** | 2.7 ± 1.2 | 1.2 ± 1.0 |
| **Hexamer** | --- | --- | 1.1 ± 0.9 | 1.9 ± 0.9 | --- | **92 ± 1.3** | 3.5 ± 1.5 |
| **Heptamer** | --- | --- | --- | --- | --- | --- | **92 ± 3** |
| **Octamer** | --- | --- | --- | 2.2 ± 0.4 | 0.1 ± 0.1 | --- | --- |
| **≥Nonamer** | --- | --- | --- | --- | 1.7 ± 1.1 | 1.8 ± 1.1 | 1.5 ± 1.2 |

## SUPPORTING FIGURE LEGENDS

**Fig. S1. [Tyr$^{1}$, Phe$^{10}$]Aβ42 oligomer stability.** [Tyr$^{1}$, Phe$^{10}$]Aβ42 was cross-linked and then electrophoresed in an SDS gel (see Methods). (a) Coomassie-stained oligomer bands were excised, re-electrophoresed, and the resulting bands visualized by silver staining. Each lane number represents the expected oligomer order. (b) Image J and MagicPlot software were used to determine the occurrence frequencies (%) of oligomers of each order. Data are representative of at least three independent experiments.

**Fig. S2. [Phe$^{10}$, Tyr$^{20}$]Aβ42 oligomer stability.** [Phe$^{10}$, Tyr$^{20}$]Aβ42 was cross-linked and then electrophoresed in an SDS gel (see Methods). (a) Coomassie-stained oligomer bands were excised, re-electrophoresed, and the resulting bands visualized by silver staining. Each lane number represents the expected oligomer order. (b) Image J and MagicPlot software were used to determine the occurrence frequencies (%) of oligomers of each order. Data are representative of at least three independent experiments.

**Fig. S3. [Phe$^{10}$, Tyr$^{30}$]Aβ42 oligomer stability.** [Phe$^{10}$, Tyr$^{30}$]Aβ42 was cross-linked and then electrophoresed in an SDS gel (see Methods). (a) Coomassie-stained oligomer bands were excised, re-electrophoresed, and the resulting bands visualized by silver staining. Each lane number represents the expected oligomer order. (b) Image J and MagicPlot software were used to determine the occurrence frequencies (%) of oligomers of each order. Data are representative of at least three independent experiments.

**Fig. S4. WT Aβ42 oligomer stability after two consecutive Cleveland gel procedures.** WT Aβ42 was cross-linked and then electrophoresed in two consecutive SDS gels (see Methods). (a) Coomassie-stained oligomer bands were excised, re-electrophoresed, and the resulting bands visualized by silver staining. Each lane number represents the expected oligomer order. (b) Image J and MagicPlot software were used to determine the occurrence frequencies (%) of oligomers of each order. Data are representative of at least three independent experiments.

**Fig. S5. [Phe$^{10}$, Tyr$^{42}$]Aβ42 oligomer stability after two consecutive Cleveland gel procedures.** [Phe$^{10}$, Tyr$^{42}$]Aβ42 was cross-linked and then electrophoresed in two consecutive SDS gels (see Methods). (a) Coomassie-stained oligomer bands were excised, re-electrophoresed, and the resulting bands visualized by silver staining. Each lane number represents the expected oligomer order. (b) Image J and MagicPlot software were used to determine the occurrence frequencies (%) of oligomers of each order. Data are representative of at least three independent experiments.

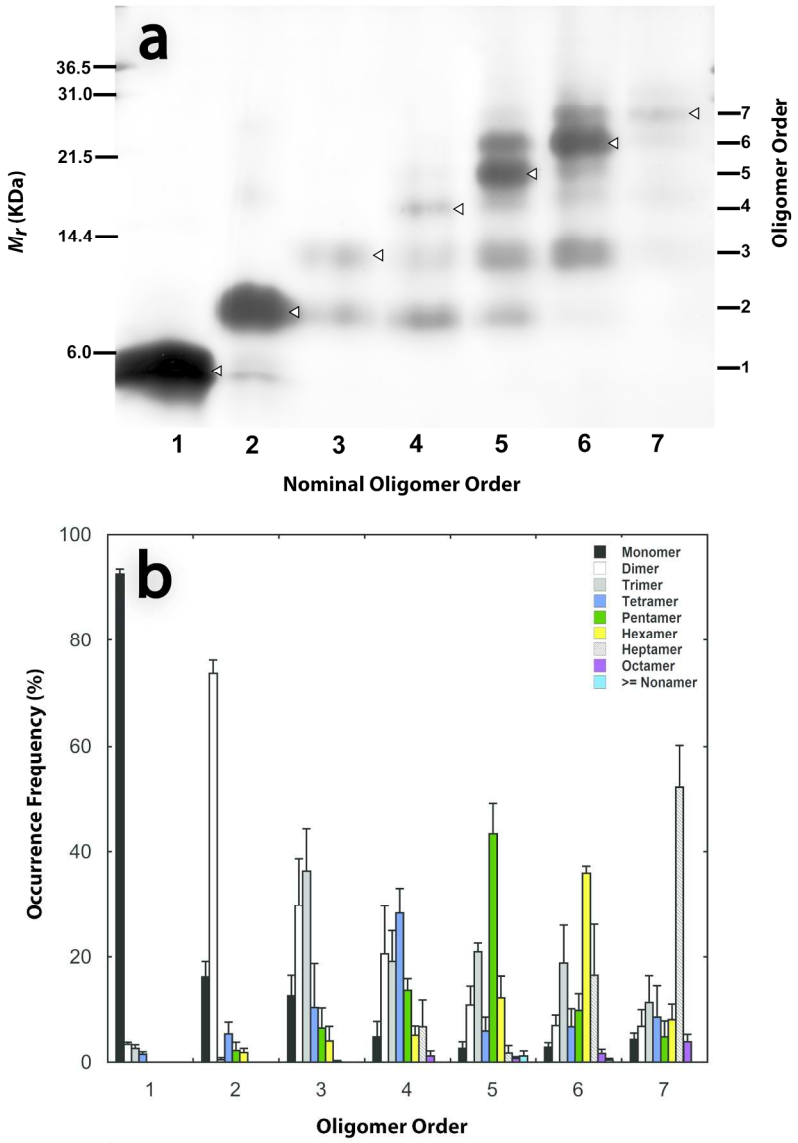


**Fig. S1**

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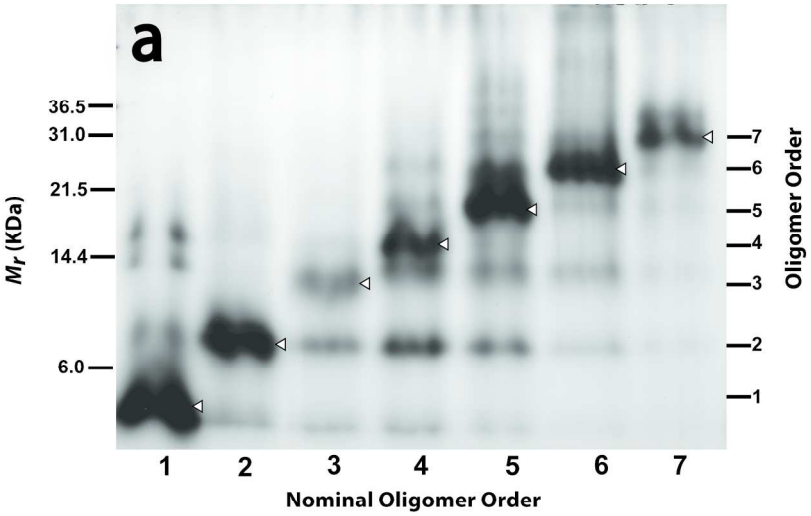


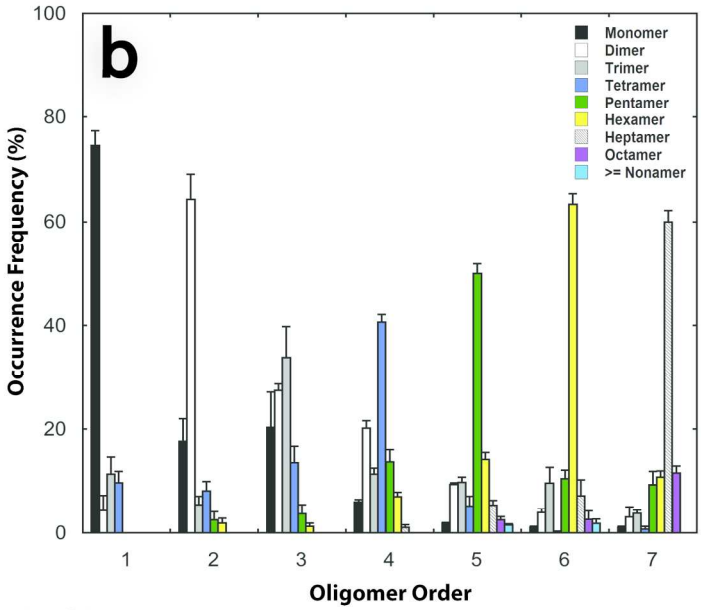


**Fig. S2**

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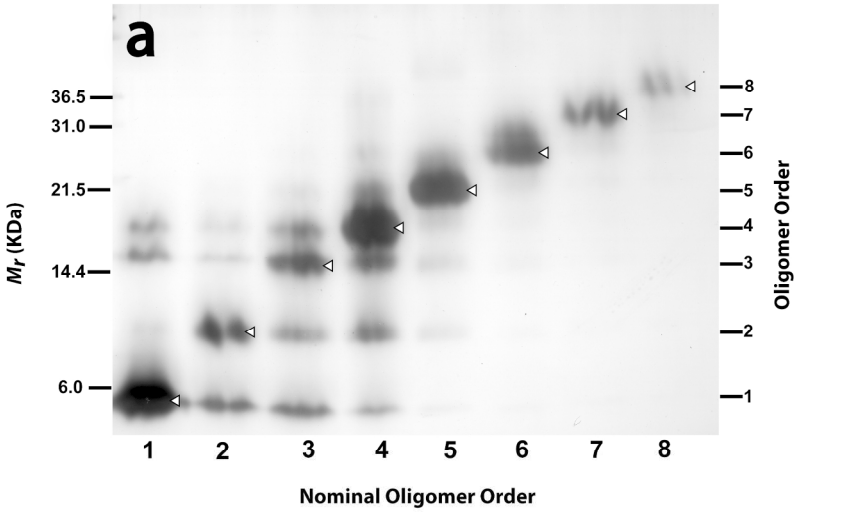


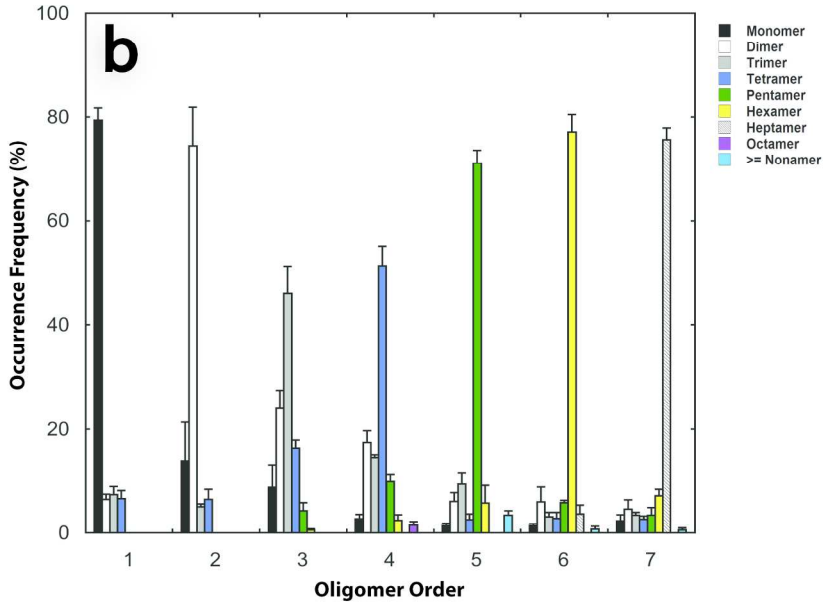


**Fig. S3**

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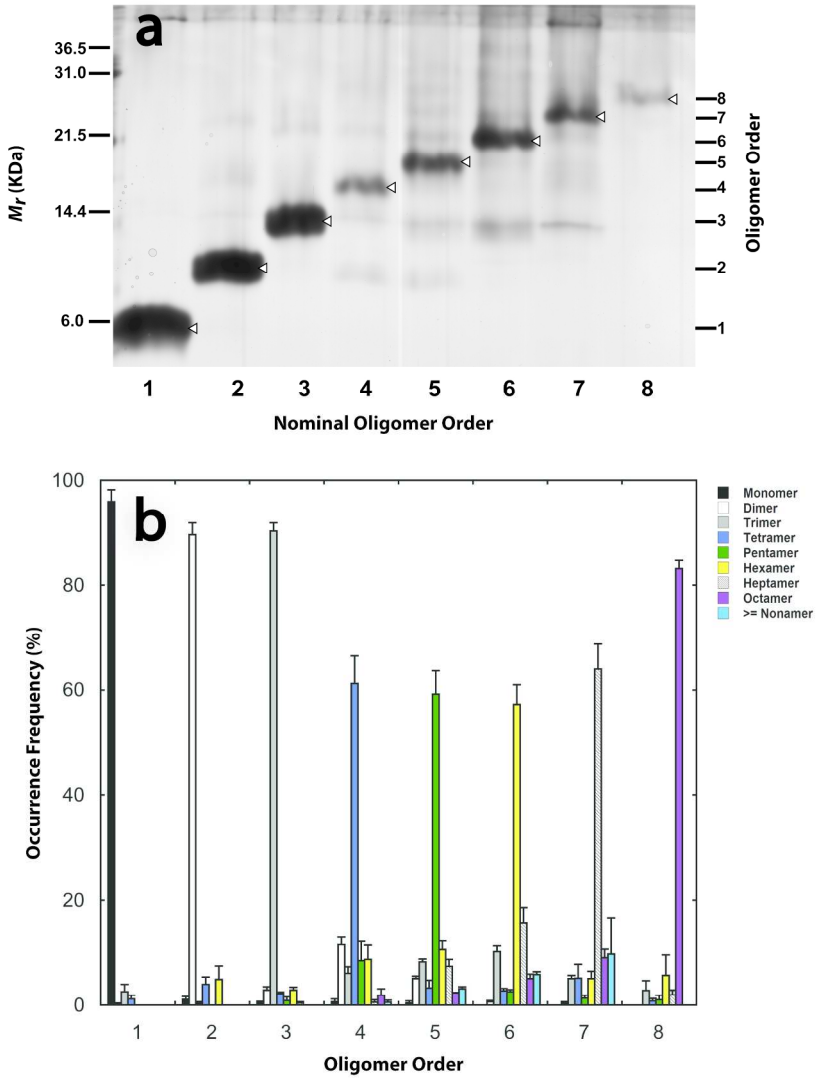


**Fig. S4**

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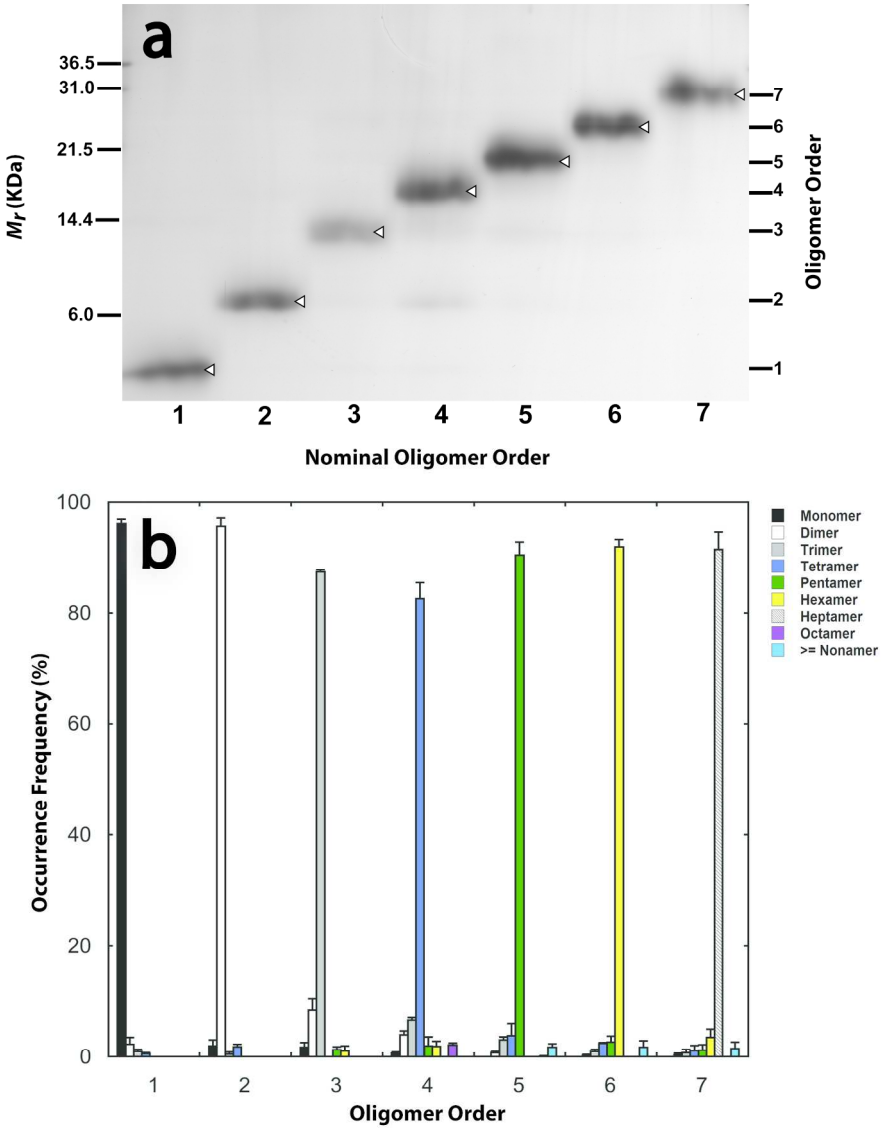


**Fig. S5**

167x227mm (300 x 300 DPI)